**REMARKS**

In the Office Action, the Examiner required restriction to one of the following inventions under 35 U.S.C. §§ 121 and 372:

Invention 1: Claims 1-7 and 26, drawn to a method for the demonstration of the occurrence of the molecular event in a cell that leads solubilization and binding of the marker protein.

Invention 2: Claims 1 and 8, drawn to a method for the demonstration of the occurrence of the molecular event in a cell that leads to cleavage or the modification of the marker protein and solubilizes it.

Invention 3: Claims 1, 9, and 27, drawn to a method for the demonstration of the occurrence of the molecular event in a cell that leads to the appearance of the subcellular anchoring fragment of the marker protein.

Invention 4: Claims 1, 10, and 14, drawn to a method for the demonstration of the occurrence of Bax activation.

Invention 5: Claims 1, 11, 12, and 14, drawn to a method for detecting the activation of a protease.

Invention 6: Claims 1, 13, and 28, drawn to a method for the demonstration of the occurrence of the molecular event in a cell that is coupled with the measurement of the cell cycle.

Invention 7: Claims 15-18, drawn to a marker protein.

Invention 8: Claims 19-22 and 29, drawn to a vector and transformed cell.

Invention 9: Claim 23, drawn to a non-human transgenic animal.

Invention 10: Claim 24, drawn to a kit comprising transformed cells or vector.

Invention 11: Claim 24, drawn to a kit comprising a transgenic animal.

Invention 12: Claim 25, drawn to a method for evaluating the activity of a candidate anti-cancer compound.

In response to the Request for Restriction, Applicants hereby elect Invention 1, corresponding to claims 1-7 and 26, drawn to a method for the demonstration of the occurrence of the molecular event in a cell that leads solubilization and binding of the marker protein. The election of Invention 1 is made with traverse. Furthermore, Applicants reserve the right to file a divisional application corresponding to the non-elected claims.

According to the Examiner, the inventions listed above as Inventions 1-12 do not relate to a single general inventive concept under PCT Rule 13.1 because they lack a special technical feature in view of Wolter *et al.* (*J. Cell Biology*, Vol. 139, No. 5, Dec. 1, 1997, pp 1281-1292) which allegedly teaches the marker protein and method recited as claim 1. Applicants respectfully disagree.

Claim 1 of the instant application relates to a method for the demonstration of the occurrence of a specific molecular event in a cell, wherein:

- the solubilization of a bound marker protein (respectively the binding of a solubilized marker protein) that is a direct or indirect marker for the occurrence of the specific molecular event is detected;
- said marker protein is present in the cell before the aforementioned detection;
- the cell, before the detection, is subjected to a permeabilization of the plasma membrane which releases the solubilized protein into the extracellular medium;

- the presence of the marker protein is then detected in the cell or in the extracellular medium by any appropriate means that make it possible to determine if solubilization, respectively binding, has occurred, and thus the corresponding molecular event.

Example 1, page 18-21 of the application as filed discloses that concerning the protein Bax, the chimeric protein obtained by the fusion of GFP to the N-terminal end of Bax allows localizing Bax. Cells transfected with a plasmid pEGFP-Bax are then exposed to the geneticin G418 (induction of apoptosis).

The protein Bax, upon activation of apoptosis, undergoes a mitochondrial relocalization.

In order to demonstrate the binding or the solubilization of Bax, the cells are then suspended in the presence of digitonin (permeabilization of the plasma membrane), and then they are analyzed by flow cytometry.

Thus, a strong drop in intensity of fluorescence is observed in cells for which Bax has not been activated, Bax-GFP being released in the extracellular medium following permeabilization. On the contrary, when Bax has been activated, the fluorescence is redistributed to mitochondria and resists permeabilization (*see*, p.20, lines 22-32).

The method defined in claim 1 thus allows analyzing, for example, if expression of a gene induces the activation of Bax or not, or evaluating the pro-apoptotic or cytoprotective capacity of a drug (*see*, Example 2, p.21, lines 21-29).

In contrast, Wolter *et al.* discloses that in cells undergoing apoptosis, the intracellular localization of the protein Bax is modified: it is shown that the protein Bax fused to a fluorescent protein (GFP-Bax) is normally soluble and is

found diffusely throughout the cytosol. After induction of apoptosis, a punctuate redistribution of GFP-Bax occurs, which is mitochondrial (*see*, abstract and page 1286, col. 2, par. *"Bax localization and mobility change during apoptosis"*).

Confocal microscopy has been used in order to analyze the mobility of the GFP-fusion proteins: in order to determine the fluorescence within a region of a cell, said region has been scanned with a laser ("*photobleaching*")(*see*, p.1282, col.2, par. *"Confocal microscopy"*).

Applicants respectfully submit that Wolter *et al.* does not disclose that the detection of the marker protein Bax is realized after permeabilization of the plasma membrane. However, it is precisely this step of permeabilization which allows demonstrating the occurrence of a specific molecular event such as apoptosis, when fluorescence after permeabilization is observed. On the contrary, when the event has not occurred, fluorescence disappears very fast.

Thus, the method according to the present invention is fast and easy to realize, and flow cytometry can be used. This approach requires a much reduced experimental manipulation compared to the technique carried out in Wolter *et al.* (*see*, page 1282, col.2, par. *"Confocal microscopy"*).

Applicants respectfully submit that the method claims 1-14 and 25-28 thus relate to the same general inventive concept, *i.e.*, the demonstration of the occurrence of a specific molecular event in a cell including a step of permeabilizing of the plasma membrane, and this concept is new in view of Wolter *et al.* Applicants see no reason for restricting these method claims into Inventions 1-6 and 12 and respectfully request reconsideration and withdrawal of the restriction requirement and rejoinder, accordingly.

If the Examiner has any questions concerning this application, he is requested to call Applicants' attorney at (908) 654-5000. If any additional fees are required by the present Communication, the Examiner is hereby authorized to charge them to our Deposit Account No. 12-1095.

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Respectfully submitted,

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